

WHAT IS CLAIMED IS:

1 1. A polymerase-nucleic acid complex, said polymerase-nucleic acid
2 complex comprising:
3 a target nucleic acid and a nucleic acid polymerase, wherein said polymerase
4 has an attachment complex comprising at least one anchor, which said at least one anchor
5 irreversibly associates said target nucleic acid with said polymerase to increase the
6 processivity index.

1 2. The polymerase-nucleic complex of claim 1, wherein said polymerase-
2 nucleic acid complex further comprises a primer nucleic acid which complements a region of
3 said target nucleic acid.

1 3. The polymerase-nucleic complex of claim 1, wherein said attachment
2 complex comprises at least two anchors.

1 4. The polymerase-nucleic complex of claim 3, wherein said attachment
2 complex is attached to a support.

1 5. The polymerase-nucleic complex of claim 1, wherein said attachment
2 complex comprises a topological tether.

1 6. The polymerase-nucleic complex of claim 3, wherein said at least two
2 anchors further comprises a topological tether.

1 7. The polymerase-nucleic complex of claim 6, wherein said topological
2 tether is attached to at least one anchor via a complementary binding pair.

1 8. The polymerase-nucleic complex of claim 6, wherein said topological
2 tether is attached to at least two anchors via at least two complementary binding pairs.

1 9. The polymerase-nucleic complex of claim 7, wherein said
2 complementary binding pairs are selected from the group consisting of any haptenic or
3 antigenic compound in combination with a corresponding antibody or binding portion or
4 fragment thereof, nonimmunological binding pairs, receptor-receptor agonist or antagonist,
5 IgG-protein A, lectin-carbohydrate, enzyme-enzyme cofactor, enzyme-enzyme-inhibitor, and
6 complementary polynucleotide pairs capable of forming nucleic acid duplexes.

1 10. The polymerase-nucleic complex of claim 9, wherein said
2 complementary binding pair is selected from the group consisting of digoxigenin and anti-
3 digoxigenin, fluorescein and anti-fluorescein, dinitrophenol and anti-dinitrophenol,
4 bromodeoxyuridine and anti-bromodeoxyuridine, mouse immunoglobulin and goat anti-
5 mouse immunoglobulin, biotin-avidin, biotin-streptavidin, thyroxine and cortisol, a
6 phenylalanine derivative and hydrazine linker and acetylcholine and receptor-acetylcholine.

1 11. The polymerase-nucleic complex of claim 1, wherein said at least one
2 anchor comprises at least one amino acid or an epitope for attachment.

1 12. The polymerase-nucleic complex of claim 11, wherein said at least one
2 amino acid is selected from the group consisting of a cysteine, a phenylalanine derivative and
3 a histidine.

1 13. The polymerase-nucleic complex of claim 12, wherein said histidine is
2 selected from the group consisting of a histidine tag, a histidine patch and a polyhistidine
3 sequence.

1 14. The polymerase-nucleic complex of claim 5, wherein said topological
2 tether comprises an antibody.

1 15. The polymerase-nucleic complex of claim 1, wherein said at least one
2 anchor is attached to a support.

1 16. The polymerase-nucleic complex of claim 1, wherein said at least one
2 anchor entraps said target nucleic acid.

1 17. The polymerase-nucleic complex of claim 6, wherein said topological
2 tether is an antibody and said at least two anchors are each a histidine tag.

1 18. The polymerase-nucleic complex of claim 1, wherein said target
2 nucleic acid is a circular DNA.

1 19. The polymerase-nucleic complex of claim 18, wherein said circular
2 DNA is sequenced by strand displacement synthesis.

1 20. The polymerase-nucleic complex of claim 1, wherein said polymerase
2 is a selected from a Family A polymerase and a Family B polymerase.

1 21. The polymerase-nucleic complex of claim 20, wherein said Family A
2 polymerase is selected from the group consisting of Klenow, Taq, and T7 polymerase.

1 22. The polymerase-nucleic complex of claim 20, wherein said Family B
2 polymerase is selected from the group consisting of a terminator polymerase, phi29, RB-69
3 and T4 polymerase.

1 23. The polymerase-nucleic complex of claim 1, wherein said polymerase-
2 nucleic acid complex is an array of polymerase-nucleic acid complexes attached to a support.

1 24. The polymerase-nucleic complex of claim 23, wherein the plurality of
2 members of said array of polymerase-nucleic acid complexes is randomly attached to said
3 support.

1 25. The polymerase-nucleic complex of claim 23, wherein the plurality of
2 members of said array of polymerase-nucleic acid complexes is uniformly attached to said
3 support.

1 26. The polymerase-nucleic complex of claim 1, wherein the processivity
2 index is at least 0.5.

1 27. The polymerase-nucleic complex of claim 26, wherein the processivity
2 index is at least 0.8.

1 28. The polymerase-nucleic complex of claim 27, wherein the processivity
2 index is 1.

1 29. A method for detecting incorporation of at least one NTP into a single
2 primer nucleic acid molecule, said method comprising:

3 i. immobilizing onto a support a polymerase nucleic acid complex
4 comprising a target nucleic acid, a primer nucleic acid which complements a region of the
5 target nucleic acid, and at least one nucleic acid polymerase;

6 ii. contacting said immobilized complex with at least one type of labeled
7 nucleotide triphosphate [NTP], wherein each NTP is labeled with a detectable label, and

8 iii. detecting the incorporation of said at least one type of labeled NTP into
9 a single molecule of said primer, while said at least one type of labeled NTP is in contact
10 with said immobilized complex, by detecting the label of the NTP while said at least one type
11 of labeled NTP is in contact with said polymerase nucleic acid complex.

1 30. The method of claim 29, wherein said polymerase nucleic acid
2 complex is contacted with a single type of labeled NTP.

1 31. The method of claim 29, wherein said polymerase nucleic acid
2 complex is contacted with at least two different types of NTPs, and wherein each type of
3 NTP is uniquely labeled.

1 32. The method of claim 29, wherein said polymerase nucleic acid
2 complex is contacted with at least four different types of NTPs, and wherein each type of
3 NTP is uniquely labeled.

1 33. The method of claim 29, wherein said NTPs are labeled on the γ -
2 phosphate.

1 34. The method of claim 33, wherein said NTPs are labeled on the γ -
2 phosphate with a fluorescent label.

1 35. The method of claim 29, wherein the detecting comprises detecting a
2 unique signal from the labeled NTP using a system or device selected from the group
3 consisting of an optical reader, a high-efficiency photon detection system, a photodiode, a
4 camera, a charge couple device, an intensified charge couple device, a near-field scanning
5 microscope, a far-field confocal microscope, a microscope that detects wide-field epi-
6 illumination, evanescent wave excitation and a total internal reflection fluorescence
7 microscope.

1 36. The method of claim 29, wherein the label of the NTP is detected using
2 a method comprising a four color evanescent wave excitation device.

1 37. The method of claim 29, wherein said detecting is carried out by a
2 mechanism selected from the group consisting of fluorescence resonance energy transfer, an
3 electron transfer mechanism, an excited-state lifetime mechanism and a ground-state complex
4 quenching mechanism.

1 **38.** The method of claim **29**, wherein said detecting step comprises
2 measuring a residence time of a labeled NTP in said polymerase nucleic acid complex.

1 **39.** The method of claim **38**, wherein the residence time of an NTP that is
2 incorporated into the primer nucleic acid is at least about 100 times longer to about 10,000
3 times longer than the residence time of an NTP that is not incorporated.

1 **40.** The method of claim **39**, wherein the residence time of an NTP that is
2 incorporated into the primer nucleic acid is at least about 200 times longer to about 500 times
3 longer than the residence time of an NTP that is not incorporated.

1 **41.** The method of claim **38**, wherein the residence time of an NTP that is
2 incorporated into the primer nucleic acid is about 1.0 milliseconds to about 100 milliseconds.

1 **42.** The method of claim **41**, wherein the residence time of an NTP that is
2 incorporated into the primer nucleic acid is about 2.0 milliseconds to about 10 milliseconds.

1 **43.** The method of claim **29**, further comprising the step of
2 genotyping said target nucleic acid by determining the identity of at least one
3 NTP that is incorporated into a single molecule of the primer.

1 **44.** The method of claim **29**, further comprising: sequencing said target
2 nucleic acid by determining the identity and sequence of incorporation of NTPs that are
3 incorporated into a single molecule of the primer.

1 **45.** The method of claim **29**, wherein said detection is a sequential
2 detection of the identities of more than one uniquely labeled dNTPs that are sequentially
3 incorporated into the primer, wherein said sequential detection yields the sequence of region
4 of the target DNA that is downstream of the elongating end of the primer.

1 **46.** The method of claim **29**, wherein said polymerase-nucleic acid
2 complex comprises a target nucleic acid and a nucleic acid polymerase, wherein said
3 polymerase has an attachment complex comprising at least one anchor, which irreversibly
4 associates said target nucleic acid with said polymerase for increasing the processivity index.